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Relationship between follicular diameter, commercial estrus breeding indicator and pregnancy rate in beef cattle

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Abstract. The objective of this study was to evaluate pregnancy rate from fixed-time artificial insemination (FTAI) performed in cows related to an estrus scores breeding indicator and measurement of preovulatory follicular diameter (POF). Forty-three crossbred Zebu cattle of reproductive age, 28-days postpartum. It was started the ovulation synchronization protocol, and eight days after the auxiliary tool for the detection of estrus (Estrotect®) was inserted. POF was measured by transrectal ultrasonographic examination. For the FTAI protocol, the animals were allocated into four groups based on POF. Group 1 (POF ≥15 mm) was inseminated immediately at ultrasound; group 2 (POF = 13–14.9 mm) 6 h later; group 3 (POF = 10–12.9 mm) 24 h; and group 4 (POF = 8–10 mm or <8 mm) 30 h. Total pregnancy rate was 72.9% (group 1: 20.1%; group 2: 20.9%; group 3: 20.9% and group 4: 16.3%). Follicles with a diameter of 7.25 mm were observed for animals with a score of 1, 11.0 mm for 2, 11.9 mm for 3, and 14.5 mm for 4. The estrus breeding indicator is a safe and practical tool, used to facilitate the reproductive management of beef cattle during the IATF protocol.

Keywords: cow, artificial insemination, ultrasonography, protocol, zebu

Relação entre diâmetro folicular, indicador de estro comercial e taxa de prenhez em gado de corte

Resumo. O objetivo deste estudo foi avaliar a taxa de prenhez por inseminação artificial em tempo fixo (IATF) realizada em vacas relacionadas a um indicador de estro e mensuração do diâmetro folicular pré-ovulatório (POF). Foram utilizadas quarenta e três vacas mestiças zebu em idade reprodutiva, 28 dias após o parto. Após 8 dias do início do protocolo de sincronização da ovulação, foi inserida a ferramenta auxiliar para detecção do estro (Estrotect®). A POF foi medida pelo exame ultrassonográfico transretal. Para o protocolo da IATF, os animais foram alocados em quatro grupos com base na POF. O grupo 1 (POF ≥15 mm) foi inseminado imediatamente à ultrassonografia; o grupo 2 (POF = 13-14,9 mm) 6h depois; grupo 3 (POF = 10–12,9 mm) 24h; e grupo 4 (POF = 8-10 mm ou <8 mm) 30h. A taxa de prenhez total foi de 72,9% (grupo 1: 20,1%; grupo 2: 20,9%; grupo 3: 20,9% e grupo 4: 16,3%). Folículos com diâmetro de 7,25 mm foram observados para animais com pontuação de 1, 11 mm para 2, 11,9 mm para 3 e 14,5 mm para 4. O indicador de estro é uma ferramenta segura e prática, utilizada para facilitar o manejo reprodutivo do gado de corte durante o protocolo de IATF.

Palavras-chave: inseminação artificial, protocolo, ultrassonografia, vaca, zebu

Introduction

Bovine market is increasingly dependent on reproductive technologies and biotechnologies, which have a major influence on its development, with artificial insemination (AI) using biotechnology the most important for genetic multiplication of the Brazilian herd (Ferreira et al., 2013; Silva & Gottschall, 2014).

AI is the mechanical deposition of semen in the female genital tract that is performed by special instruments and has become an important method to aid in the breeding and genetic multiplication of the Brazilian herd (Mies Filho, 1970; Prado et al., 2015). Fixed time artificial insemination (FTAI) is a biotechnology that has emerged to solve the difficulties with conventional AI because there is no requirement to observe estrus or to decrease the period of anestrus post calving; thus, increasing the pregnancy rate of the herd (Bonato et al., 2012). FTAI is performed through protocols of estrus synchronization and ovulation in a batch of females, making it possible to inseminate a larger number of animals in a shorter period (Martins et al., 2009; Morotti et al., 2013). FTAI has shown pregnancy rates of 40% - 60%, making this biotechnology the most successful biotechnology in beef cattle in Brazil (Bonato et al., 2012).

FTAI protocols are performed with the application of hormones that control the estrus cycle and ovulation of females. Currently, there are a variety of protocols for FTAI. However, the ovulation-inducing hormone used, dose, quality, and timing of the application directly determine the success of this technique and influence the fertility of the animals. It is important to observe the diameter of the dominant follicle at the time of FTAI because it is directly related to oocyte follicular maturation (Pfeifer et al., 2015). FTAI ensures that the producer chooses a particular time to inseminate a larger number of animals without the need to wait for the exact time of estrus for each animal, thus optimizing the work of the veterinarian and increasing farm productivity (Inforzato et al., 2008).

There are still some limitations of this technique, including the use of specialized labor required to identify animals in heat, the adequate structure of the farm, and purchase of semen storage equipment and instruments for insemination (Mies Filho, 1970; Prado et al., 2015). The constant training of the veterinarian and their commitment activities are of fundamental importance to the success of the total pregnancy rates (Martins et al., 2009; Morotti et al., 2013).

There are a variety of hormonal protocols for performing FTAI with the goal of inducing and synchronizing estrus and ovulation. However, it is fundamental that the veterinarian carefully evaluates each situation so the most appropriate measures are used for each farm (Quirino et al., 2019). Hormones are the main objects of the study of the reproduction of herds because a suitable protocol allows a higher success in pregnancy rate (Câmara et al., 2009; Inforzato et al., 2008; Prado et al., 2015; Silva & Gottschall, 2014).

The advantages of FTAI include the insemination of females at a predetermined moment, increased fertility, induction of cow cyclicity in anestrus, increased pregnancy rate, evaluation of the hormonal response of females before AI, and reduction of the interval between calving. In addition, it is possible to evaluate the fertility of animals in a given batch via the diagnosis of ovarian diseases or to discard animals that did not respond to the synchronization treatment (Pfeifer et al., 2015).

Currently, there are commercial products that can assist in the detection of heat without the need for continuous observation of the animals. Estrotect® is a device that assists in the detection of estrus, which consists of a color-changing adhesive based on the number of times that the animal accepts it, demonstrating the proximity of estrus (Bonato et al., 2012). Based on the observations of the device, it is possible to measure how close to estrus the animal is, with the closest being the area that shows a fluorescent orange coloration (Bonato et al., 2012).

The objective of the present study was to evaluate the pregnancy rate of cross-breeding Zebu cows with FTAI performed in groups related to the estrus breeding indicator and preovulatory follicular diameter.

Material and methods

This research was approved by the Animal Ethics and Research Ethics Committee on the Use of Animals (ECUA), Unisalesiano Araçatuba, number 37/2017.

The present study included 43 crossbred Zebu cows of reproductive age (24 to 36 months), 28 days post calving and body condition score between 3 and 3.5. The animals were kept in semi-confinement and fed with silage and pasture water *ad libitum*, as well as mineral salt.

The protocol of synchronization of ovulation to FTAI was performed as follows: 1) on the first day (day 0), application of 2 mg of estradiol benzoate intramuscularly and the placement of intravaginal device containing 1.9 g of progesterone; 2) after 8 days (day 8), application of 12.5 mg of prostaglandin F2α4 intramuscularly; withdrawal of the intravaginal device and application of 300 IU of equine chorionic gonadotropin intramuscularly, and implantation of the device for the detection of estrus (Estrotect®[1]), which adhered transversely to the vertebral column near the transitional region of the sacral loins; 3) after 24 h (day 9), application of 1 mg of estradiol benzoate; and 4) on day 10, the estrus scores breeding indicator Estrotect® was evaluated and classification was modified according to the manufacturer's instructions (a score from 1 to 4), follicular measurement, and placed in groups based on the diameter of the preovulatory follicle (Figure 1).

Figure 1. Evaluation of crossbred Zebu cows submitted to fixed time artificial insemination, estrus synchronization, and evaluation of estrus detectors protocols.

The preovulatory follicle diameter (POF) was measured by transrectal ultrasonographic examination using a linear transducer of 5 to 7.5 MHz. After that the animals were divided into four groups based on the follicular diameter and FTAI was performed at times predetermined by Pfeifer et al. (2015), Table 1.

Table 1. Groups and time classification of insemination according to follicular diameter

Groups	Follicle diameter (mm)	Time of Insemination, post ultrasound
1	≥ 15	Immediately
2	13 - 14.9	6h after
3	10 - 12.9	24h after
4	< 10	30h after

Estrus scores breeding indicator were evaluated based on the change in color because of the amount of friction present during acceptance or non-acceptance during estrus. This change in color was divided into a score based on the variation in the placement of the device, classified scores from 1 to 4, according manufacture's protocols. Score 1 referred to an animal that did not accept mounts, score 2 indicated animals that accepted from 1 to 3 mounts, score 3 animals that accepted from 3 to 5 mounts, and score 4 indicated an animal that accepted more than 5 mounts (Bonato et al., 2012). After 30 days of FTAI, the animals were submitted to ultrasonographic evaluation to diagnosis gestation. The animals that presented negative pregnancy were resubmitted to resynchronization and FTAI in groups. After that, the animals were again submitted to ultrasound examination for pregnancy detection.

Results were analyzed with the GLIMMIX or MIXED procedures of the SAS program (SAS Institute Inc., Cary, NC), using the cow as the experimental unit. Significance was established as $P \leq 0.05$ and trends were reported if $P > 0.05$ and ≤ 0.10.

Results and discussion

The pregnancy rates were 8.8% (4/43) of the animals had a score of 1, 11.8% (5/43) had a score of 2, 32.4% (14/43) had a score of 3, and 47.1% (20/43) had a score of 4 using the Estrotect®, demonstrating that the higher the Estrotect® score, the higher the pregnancy rate from FTAI (Figure 2). The total pregnancy rate was 72.9% (31/43) from the FTAI protocol used in the present study, presenting a higher value than that found in the literature.

Bonato et al. (2012) cited a pregnancy rate of 40%–60% using a conventional FTAI technique. According to Ribeiro Filho et al. (2013), Estrotect® can determine the ovulation response of females submitted to FTAI protocols. The data obtained from the present study showed that the ovulation response was directly related to the estrus scores breeding indicator because animals with higher scores had higher conception rates. Ribeiro Filho et al. (2013) affirmed that the diameter of the ovulatory follicle in the FTAI programs was a determinant for improvement in conception rates and reproductive efficiency of the herd.

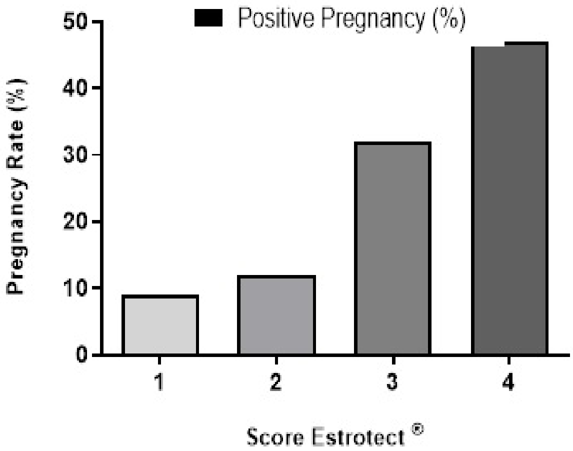

Figure 2. Pregnancy rates of Zebu crossbred cows related to estrus detection scores using the Estrotect® device.

From the 43 animals evaluated, 10 (23.3%) animals presented POF greater than 15 mm and were allocated to group 1, 11 (25.6%) had POF between 13.0 and 14.9 mm and were allocated in group 2, 11 (25.6%) had POF between 10.1 and 12.9 mm and were placed in group 3, and 13 (30,2%) had POF between 8 and 10 mm or lower than 8 mm and were placed in group 4.

According to Vasconcelos et al. (2001), females with smaller diameter follicles had lower concentrations of estradiol at the time of ovulation compared to females with larger follicles or when ovulation occurs spontaneously. Thus, the high concentration of estradiol in the preovulatory phase because of the greater follicular diameter favored conception, that is, the greater the diameter of the ovulatory follicle, the greater the size of the corpus luteum, and consequently, the higher the conception rate. The values follicular diameter in crossbred cows in the present study, where the animals had a mean POF of 12.44 mm are similarly to mean previously by Ribeiro Filho et al. (2013) (12.43 mm).

The division of the animals into groups allowed us to determine the most appropriate moment for insemination. Although Pfeifer et al. (2015) stated that animals with a POF less than 8 mm should not be inseminated, it was decided to inseminate these animals at the same time as those with follicles of 8 to 10 mm; that is, 30 h after ultrasound examination.

Evaluating the pregnancy rate related to the mean follicular diameter of the groups, the results showed a pregnancy rate of 23.4% for the animals in group 1, 23.3% for group 2, 23.3% for group 3, and 30.2% for group 4. Thus, the greater the diameter of the ovulatory follicle, the greater its ovulatory capacity; this may explain the higher design rate in females with larger follicular diameter.

In the study by Ribeiro Filho et al. (2013), the animals were categorized into three groups according to the POF, where females that presented POF above 13.60 mm had an estimated mean conception probability of 78.8%, which was significantly higher than those with diameters of up to 11.20 mm or between 11.30 and 13.60 mm, whose values were 33.8% and 59.2%, respectively. Corroborating the results of Ribeiro Filho et al. (2013), Zebu cows submitted to FTAI protocols had satisfactory conception indices if the follicle diameter was larger at the time of FTAI.

Perry et al. (2007) showed a significant effect of higher follicular diameter at the time of FTAI on the probability of conception in Nellore heifers. Ovulation of follicles with diameters less than 11 mm resulted in an increase in the percentage of embryonic loss and fetal mortality in beef cows synchronized with the presence of a larger diameter follicle at the time of insemination. Thus, alternatives should be adopted to increase the diameter of the follicle at the time of FTAI; thus, increasing the efficiency of the synchronization protocols and providing a better cost-benefit ratio for the producer.

When we compared the mean diameter of the POFs to the score of the estrus scores breeding indicator (Estrotect®) we observed a follicle with a diameter of 7.25 mm for animals with a score of 1; 11 mm with a score of 2; 11.88 mm with a score of 3 and 14.52 mm with a score of 4 (Figure 3). Thus, the follicular diameter was directly related to the ovulatory response of animals. The higher the ovulation, the greater the score of the device, and consequently, the greater the follicular diameter.

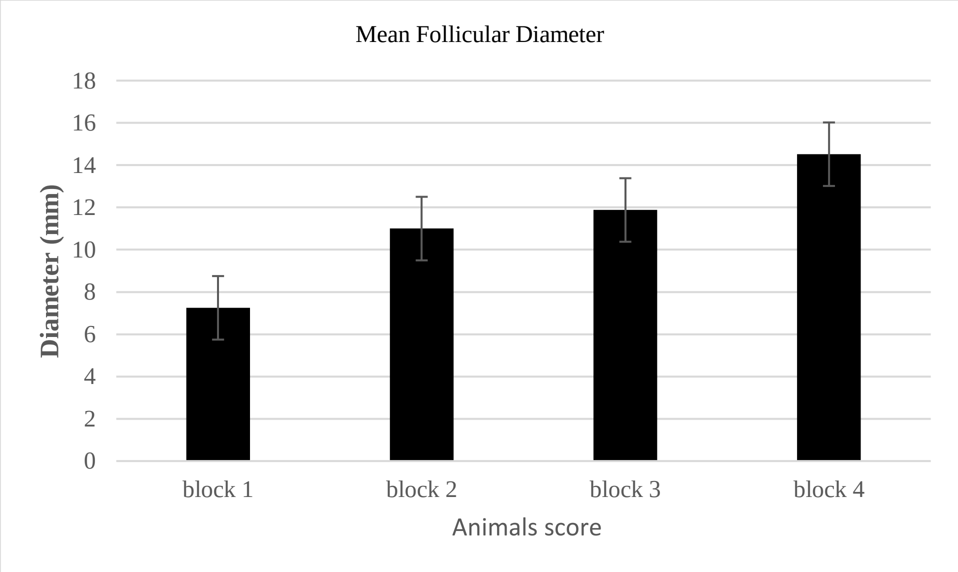

Figure 3. Mean follicular diameters of the preovulatory follicles in Zebu crossbred cows related to the estrus detection using the Estrotect® device.

Conclusion

From the results obtained in the present study, the Estrotect® score was directly related to a larger follicular diameter, and consequently, an increased pregnancy rate. Thus, the estrus breeding indicator is a useful tool to assist in FTAI protocols without the need for follicular or behavioral evaluation and can be used to facilitate the reproductive management of cattle.

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Conflict of interest statement

The authors of this work declare that there is no conflict of interest.

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